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Enzymes and Protein Markers

Cardiac Troponins I and T Are Biological Markers of Left Ventricular Dysfunction in Septic Shock

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Background: **Cardiac depression in severe sepsis and septic shock is characterized by left ventricular (LV) failure. To date, it is unclear whether clinically unrecognized myocardial cell injury accompanies, causes, or results from this decreased cardiac performance. We therefore studied the relationship between cardiac troponin I (cTnI) and T (cTnT) and LV dysfunction in early septic shock.**

Methods: **Forty-six patients were consecutively enrolled, fluid-resuscitated, and treated with catecholamines. Cardiac markers were measured at study entry and after 24 and 48 h. LV function was assessed by two-dimensional transesophageal echocardiography.**

Results: **Increased plasma concentrations of cTnI (≥0.4 μg/L) and cTnT (≥0.1 μg/L) were found in 50% and 36%, respectively, of the patients at one or more time points. cTnI and cTnT were significantly correlated ($r = 0.847$; $P < 0.0001$). Compared with cTnI-negative patients, cTnI-positive subjects were older, presented higher Acute Physiology and Chronic Health Evaluation II scores at diagnosis, and tended to have a worse survival rate and a more frequent history of arterial hypertension or previous myocardial infarction. In contrast, the two groups did not differ in type of infection or pathogen, or in dose and type of catecholamine administered. Continuous electrocardiographic monitoring in all patients and autopsy in 12 nonsurvivors did not disclose the occurrence of acute ischemia during the first 48 h of observation. LV dysfunction was strongly associated with cTnI positivity (78% vs 9% in cTnI-negative patients; $P < 0.001$). In multiple regression analysis, both cTnI and cTnT were exclusively associated with LV dysfunction ($P < 0.0001$).**

Conclusions: **These findings suggest that in septic shock, clinically unrecognized myocardial cell injury is a marker of LV dysfunction. The latter condition tends to occur more often in severely ill older patients with underlying cardiovascular disease. Further studies are needed to determine the extent to which myocardial damage is a cause or a consequence of LV dysfunction.**

Inadequate myocardial performance, characterized by left ventricular (LV)[4] systolic depression and diastolic dilatation, is a common and early complication of septic shock *(1, 2)*. Studies in humans *(3, 4)* strongly argue against an ischemic origin of sepsis-induced cardiac injury. However, a dysfunctional microcirculation that produces regional flow disturbances and abnormal tissue oxygenation is a hallmark of septic shock, which may cause relative ischemia in various organs, including the heart *(5, 6)*. Moreover, regional myocardial ischemia may well be present in septic patients with identifiable coronary risk factors or coexistent coronary artery disease.

Cardiac troponins I (cTnI) and T (cTnT) are cardiospecific markers of prognostic value in acute myocardial infarction (AMI) *(7–9)*, unstable angina *(10, 11)*, acute chest pain *(12–15)* myocarditis *(16)*, cardiac trauma *(17)*, and perioperative cardiac complications *(18)*. Recently, cTn positivity has been documented in patients with heart failure of nonmyocardial ischemic origin *(19, 20)* and in a heterogeneous population of critically ill patients in medical *(21, 22)*, surgical *(23)*, and pediatric *(24)* intensive care units (ICUs). Increased cTn concentrations have been described in the plasma of patients with sepsis *(22)* and septic shock *(25, 26)* in association with an increased mortality.

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[4] Nonstandard abbreviations: LV, left ventricular; cTn, cardiac troponin; AMI, acute myocardial infarction; ICU, intensive care unit; CK-MB, creatine kinase MB isoenzyme; CRP, C-reactive protein; TEE, two-dimensional transesophageal echocardiography; APACHE II, the Acute Physiology and Chronic Health Evaluation II; ECG, electrocardiography; and P_c, P value corrected for the number of comparisons (Bonferroni adjustment).

The pathophysiological mechanism; the clinical, functional, and biochemical correlates; and the prognostic significance of increased cTn concentrations in septic shock remain poorly understood. Therefore, a cohort of patients with early fully resuscitated septic shock was prospectively studied with the following aims: (*a*) to determine the prevalence of increased cTnI and cTnT in septic shock; (*b*) to compare cTn-positive and -negative patients in terms of demographic data, clinical presentation, outcome, presence of other biochemical markers of cardiac injury [creatine kinase MB isoenzyme (CK-MB) mass determination; EC 2.7.3.2] or sepsis [procalcitonin and C-reactive protein (CRP)] *(27, 28)*, and anatomopathological findings in nonsurvivors; (*c*) to correlate cTn concentrations with in vivo LV dysfunction as assessed by two-dimensional transesophageal echocardiography (TEE).

Materials and Methods

PATIENTS

The study was approved by the Committee for Ethics in Human Research of the Academic Hospital of the Vrije Universiteit Brussel. Informed consent was obtained from the next of kin of each patient. Forty-six patients (ages, 18–93 years; median, 66 years; male-to-female ratio, 30/16) in whom septic shock was diagnosed within 4 h of clinical onset were consecutively enrolled. Septic shock was defined according to consensus guidelines *(29)* as sepsis with hypotension unresponsive to fluid resuscitation and evidence of organ hypoperfusion or dysfunction, namely, (*a*) hypotension, defined as systolic pressure <90 mmHg, or reduced from baseline by >40 mmHg; and (*b*) two or more of the following criteria: temperature >38 °C or <36 °C; heart rate >90 beats/min; respiratory rate >30/min or hyperventilation with Pco_2 <32 mmHg; white blood cell count >12 000/μL, <4000/μL, or >10% immature cells.

Exclusion criteria included the presence of any cardiothoracic event within 1 month before inclusion (coronary insufficiency, cardiothoracic trauma or surgery, cardiopulmonary resuscitation, cardioversion, or endo-, myo-, or pericarditis) and immunosuppressed state [treatment with steroids, bone marrow or organ transplant recipients, leukopenia (white blood cell count <1000/μL) or neutropenia (polymorphonuclear granulocyte count <500/μL), hematological malignancy, and AIDS], and a medical condition considered to be irreversible or lethal within 24 h after admission.

All patients had indwelling radial (arterial line kit; Argon) and balloon-tipped pulmonary artery catheters (Edwards Swan-Ganz model 97-120-7F; Baxter Healthcare) and, if required, were mechanically ventilated in volume- or pressure-controlled modes (Servo 900C ventilator; Siemens Elema) under continuous analgesic sedation with midazolam and fentanyl. All patients received routine resuscitation therapy for septic shock, including fluid administration with crystalloids and colloids, dobutamine to maintain cardiac index $\geq 4\ L \cdot min^{-1} \cdot m^{-2}$, and dopamine and/or norepinephrine to maintain mean arterial pressure >65 mmHg. After blood and various biological specimens were collected for microbiological analysis, all patients initially received broad-spectrum antibiotics consisting of a combination of an aminoglycoside with either a fourth-generation cephalosporin or ciprofloxacin. Antibiotic treatment was adjusted based on culture results.

CLINICAL AND FUNCTIONAL INVESTIGATIONS

The Acute Physiology And Chronic Health Evaluation (APACHE) II score *(30)* was used to determine the initial severity of illness. An inotrope score *(31)* was used to adjust for relative catecholamine dependency. This score took into account the type and dose of adrenergic agent(s) used and was obtained for each patient after the desired endpoints for resuscitation were achieved. When a patient received a combination of adrenergic drugs, the inotrope score was calculated as the sum of the scores for each individual agent. Any histories of cardiomyopathy, AMI, arterial hypertension, diabetes mellitus, or peripheral arteriopathy were retrieved from the patients' medical files.

After hemodynamic stabilization, a baseline 12-lead electrocardiography (ECG) and a TEE were performed. Heart rate and rhythm were continuously monitored. ECG tracings were printed every 4 h and whenever arrhythmias occurred. ECG findings matching myocardial ischemia were defined as flattened, inverted, or abnormally tall T-waves; a horizontal or sloping S-T segment depression; or a ST-segment elevation. LV dysfunction was defined echocardiographically as the concomitant presence of an increased LV end-diastolic diameter (>60 mm) and volume (>120 cm^3), the presence of regional and global LV hypokinesia, and a LV fractional area contraction of <0.4 under inotropic support *(32)*. The same investigator (D.N.N.) performed all TEE measurements and assessed all ECG tracings while being blinded to the results for the cardiac markers.

ANATOMOPATHOLOGICAL OBSERVATIONS

Survival was defined as leaving the hospital alive and able to resume all previous daily activities. Whenever possible, immediate postmortem examinations were performed in nonsurvivors to obtain myocardial tissue for light microscopy examination. One fragment of LV free wall was fixed in 100 mL/L formalin, embedded in paraffin, and stained with hematoxylin-eosin, Masson trichrome, and Congo red. Sarcoplasmic fibrils and contraction band necrosis were studied with a peroxidase technique (Dako Envision Systems) using a desmin antibody (monoclonal anti-desmin II, 53-kDa desmin protein specificity; ICN Pharmaceuticals) *(33)* and with Heidenhain's iron hematoxylin, respectively *(33, 34)*. All samples were assessed by the same pathologist (C.G.) blinded to the results of the cardiac markers.

BLOOD SAMPLING AND BIOCHEMICAL ASSAYS

Arterial blood samples were collected in lithium-heparin Monovettes (Sarstedt) on admission at the ICU and after

24 and 48 h. Samples were centrifuged immediately at 3000*g* for 10 min (Hettich Zentrifugen), and the plasma was aliquoted and stored at −70 °C. cTnI was measured by the Stratus II cTnI fluorometric enzyme immunoassay (Dade Behring) *(35, 36)*, cTnT by the Elecsys 2010 Troponin T STAT immunoassay (second-generation immunoglobulins; Roche), CK-MB mass by the Abbott AxSYM CK-MB microparticle enzyme immunoassay (Abbott Laboratories), procalcitonin by the LUMItest PCT immunoluminometric assay (BRAHMS Diagnostica), and CRP by the Vitros CRP slide enzymatic immunoassay (Johnson & Johnson Clinical Diagnostics). Cutoff values considered indicative of cardiac injury when equaled or exceeded were 0.4 μg/L for cTnI, 0.1 μg/L for cTnT, and 9.0 μg/L for CK-MB mass. Cutoff values for positivity were 0.5 μg/L for procalcitonin, and 10 mg/L for CRP.

STATISTICAL ANALYSIS

Statistical tests were performed two-tailed using the GraphPad Prism, Ver. 2.0 (GraphPad Software). Differences in prevalences and medians were determined by the two-tailed Fisher exact test and the Mann–Whitney *U*-test, respectively, and were considered significant at $P < 0.05$. In case of k comparisons, a corrected P value (P_c) was computed by multiplying the P value by a factor k (Bonferroni adjustment). Differences in diagnostic information conferred by cTnI and cTnT status were assessed two-tailed by the McNemar exact test for paired observations. Nonparametric correlations between two variables were calculated by Spearman correlation. Multiple linear regression analysis (Windows 8.0; SPSS) assessed the ability of cTnI and cTnT concentrations to independently predict LV dysfunction after adjustment for other variables. $P < 0.05$ was considered statistically significant.

Results

CAUSES AND OUTCOME OF SEPSIS

We prospectively followed 46 consecutively recruited patients with septic shock. Pneumonia was the most frequent underlying cause, followed by peritonitis and urosepsis (Table 1). Most infections (29 of 46, or 63%) were caused by gram-negative bacteria alone or in combination with other pathogens (Table 1). In 39 patients (85%), one or more pathogenic infectious agents could be isolated or identified (Table 1). In the remaining seven patients, no microorganisms could be cultured, but in all of them the infectious focus was confirmed either clinically or at autopsy. Survivors (n = 25) and nonsurvivors (n = 21) did not differ in terms of underlying etiology of septic shock (Table 1) or sex ratio (Table 2). Nonsurvivors were older than survivors and tended (significance lost after Bonferroni correction) to have higher APACHE II and inotrope scores. The two groups did not differ in type or dose of specific catecholamines administered or in a previous medical history of AMI or arterial hypertension (Table 2).

Table 1. Origin of septic shock and microbiological findings.

	Patients with septic shock, n		
Septic shock	All (n = 46)	Survivors (n = 25)	Nonsurvivors (n = 21)
Type of infection			
Pneumonia[a]	34	17	17
Peritonitis[b]	6	4	2
Urosepsis[c]	3	2	1
Wound infection[d]	1	1	
Catheter sepsis[c]	1		1
Meningitis[c]	1	1	
Type of pathogen			
Gram-negative	22	13	9
Gram-positive	6	4	2
Mixed (≥1 type of gram-negative)	7	4	3
Anaerobe	1	1	
Viral	2	1	1
Yeast	1		1
Not identified	7	2	5

[a] The diagnosis of pneumonia was based on clinical and chest x-ray findings in all patients. Twenty patients grew pathogens on quantitative culturing of endobronchial secretions obtained by bronchoalveolar lavage or protected specimen brush, 13 patients had similar microorganisms in bronchial aspirate and blood cultures, and in 1 patient, pneumonia was confirmed at autopsy.

[b] Peritonitis was diagnosed preoperatively in all patients, two of them also having positive blood cultures.

[c] Blood cultures were positive in patients with catheter sepsis, urosepsis, and meningitis.

[d] The diagnosis of wound infection was made clinically.

CARDIAC MARKERS IN SEPTIC SHOCK: CLINICAL, FUNCTIONAL, AND BIOLOGICAL CORRELATES

A high prevalence of increased cardiac markers (cTnI, cTnT, and CK-MB mass) was observed on admission at the ICU and during the next 2 days. cTnT, cTnI, and CK-MB mass concentrations were above the cutoffs in 50%, 36%, and 41% of the patients, respectively, at one or more time points (Table 3). In marker-positive patients, the peak concentrations (median, interquartile range) were 1.4 μg/L (0.8–6.8 μg/L) for cTnI (n = 23); 0.66 μg/L (0.19–1.51 μg/L) for cTnT (n = 16); and 20.3 μg/L (10.9–37.1 μg/L) for CK-MB mass (n = 19). A close correlation existed between cTnI and cTnT values ($r = 0.847$; $P < 0.0001$); a less strong but still significant correlation was found between cTnI and CK-MB mass ($r = 0.618$; $P < 0.0001$) and between cTnT and CK-MB mass concentrations ($r = 0.525$; $P < 0.0001$; Fig. 1). The temporal changes of cTnI plasma concentrations did not differ meaningfully in survivors compared with nonsurviving patients (data not shown). Overall, the prevalence of increased cTn or CK-MB concentrations, if anything, tended to decrease with observation time (Table 3).

cTnI-positive and -negative patients were next compared in terms of demographic, clinical, and biological markers (Table 4). The male-to-female ratio did not differ according to cTnI status. cTnI-positive patients were on average older, had higher APACHE II scores on admission, and tended

Table 2. Patient characteristics.

Characteristics	All (n = 46)	Survivors (n = 25)	Nonsurvivors (n = 21)	*P*
Demographic				
Male-to-female ratio	30/16	18/7	12/9	NS[a,b]
Age, median (interquartile range), years	66 (54–74)	54 (49–68)	72 (66–75)	0.001[c,d]
Clinical				
APACHE II score, median (interquartile range)	24 (20–30)	21 (18–24)	27 (22–30)	0.018[c,e]
LV dysfunction, n (%)	20 (43)	8 (32)	12 (57)	NS[b]
Inotrope score, median (interquartile range)	4 (2–6)	3 (2–4)	6 (3–7)	0.032[c,e]
Catecholamine dose,[f] median (interquartile range), $\mu g \cdot kg^{-1} \cdot min^{-1}$				
Dopamine	3 (0–10)	0 (0–5)	5 (0–10)	NS[c]
Dobutamine	5 (0–10)	5 (0–5)	10 (0–15)	NS[c]
Norepinephrine	0.1 (0.0–0.3)	0.1 (0.0–0.3)	0.2 (0.0–0.5)	NS[c]
Medical history, n (%)				
AMI	10 (22)	4 (16)	6 (28)	NS[b]
Arterial hypertension	13 (28)	5 (24)	8 (38)	NS[b]

[a] NS, not significant.
[b] Fisher's exact test used for assessing differences between survivors and nonsurvivors.
[c] Mann–Whitney *U*-test used for assessing differences between survivors and nonsurvivors.
[d] $P_c = 0.01$ after correction for the number of comparisons (n = 10; Bonferroni adjustment).
[e] P_c, not significant after correction for the number of comparisons (n = 10; Bonferroni adjustment).
[f] At the time of TEE.

(significance lost after Bonferroni correction) to have a worse outcome in terms of survival and to present more often with a history of AMI and arterial hypertension (Table 4), but not of diabetic arteriopathy or cardiomyopathy (data not shown). A significant correlation was found between APACHE II score on admission and peak cTn concentrations ($P = 0.0004$ for cTnI, and $P = 0.001$ for cTnT by Spearman correlation). The inotrope score, dose of specific catecholamines administered, and gram-negative origin of shock were similar in both groups (Table 4). Regardless of cTnI status, atrial fibrillation, atrial flutter, and supraventricular tachycardia were frequently documented (data not shown). However, none of the patients presented signs of acute ischemia on ECG during the first 48 h after inclusion in the study (Table 4). TEE disclosed a LV dysfunction in 18 (78%) of cTnI-positive patients but in only 2 (9%) cTnI-negative patients ($P < 0.0001$; Table 4).

All cTnI-negative patients were also cTnT negative, but seven subjects who were cTnI positive at some time point remained cTnT negative ($P < 0.03$ by McNemar's exact test). CK-MB mass did not occur more frequently in cTnI-positive compared with cTnI-negative patients (Table 4). Markers of severity of septic shock, such as procalcitonin *(27)* and CRP *(28)*, were positive on admission in 91% and 98% of cases, respectively, and became positive in all patients after 24 h. Overall, cTnI-positive and -negative patients did not differ in procalcitonin and CRP concentrations (Table 4). In survivors, procalcitonin concentrations after 24 h were similar to those on admission but tended to decrease after 48 h ($P < 0.01$; Friedman-test). In nonsurvivors, procalcitonin and CRP values did not change significantly after 48 h. Clinical and biochemical differences between cTnT-positive and -negative patients were similar to, albeit somewhat less pronounced than, those found between cTnI-positive and -negative subjects (data not shown).

After the distribution of maximal cTnI concentrations was normalized by logarithmic transformation, multiple linear regression analysis was used to assess the independent predictor ability of cTnI for LV dysfunction after adjustment for the following independent variables: age, gender, survival, APACHE II and inotrope scores, dose of norepinephrine administered, and history of AMI and arterial hypertension. A highly significant association ($P < 0.0001$) between cTnI values and LV dysfunction on TEE was found. After adjustment for LV dysfunction, no significant correlation between cTnI concentrations and any of the other variables tested was observed even after the number of variables was reduced to five (LV dysfunction, age, survival, APACHE II score, norepinephrine dose). Similar results were found when cTnT concentrations were log-transformed and analyzed in the same way (data not shown).

Table 3. Prevalence of cardiac markers.[a]

Time point	cTnI[b]	cTnT[c]	CK-MB mass[d]
Admission	18/46 (39)	14/42 (33)	13/46 (28)
24 h after inclusion	20/45 (44)	13/40 (32)	13/45 (29)
48 h after inclusion	11/41 (27)	11/34 (32)	5/41 (12)
≥1 time point	23/46 (50)	16/45 (36)	19/46 (41)
≥2 time points	17/45 (38)	15/41 (37)	11/45 (24)

[a] Values given as n (%).
[b–d] Cutoff values for positivity: [b] cTnI ≥0.4 μg/L; [c] cTnT ≥0.1 μg/L; [d] CK-MB mass ≥9.0 μg/L. Missing data attributable to insufficient serum sample or to death of patient within 48 h after onset of septic shock.

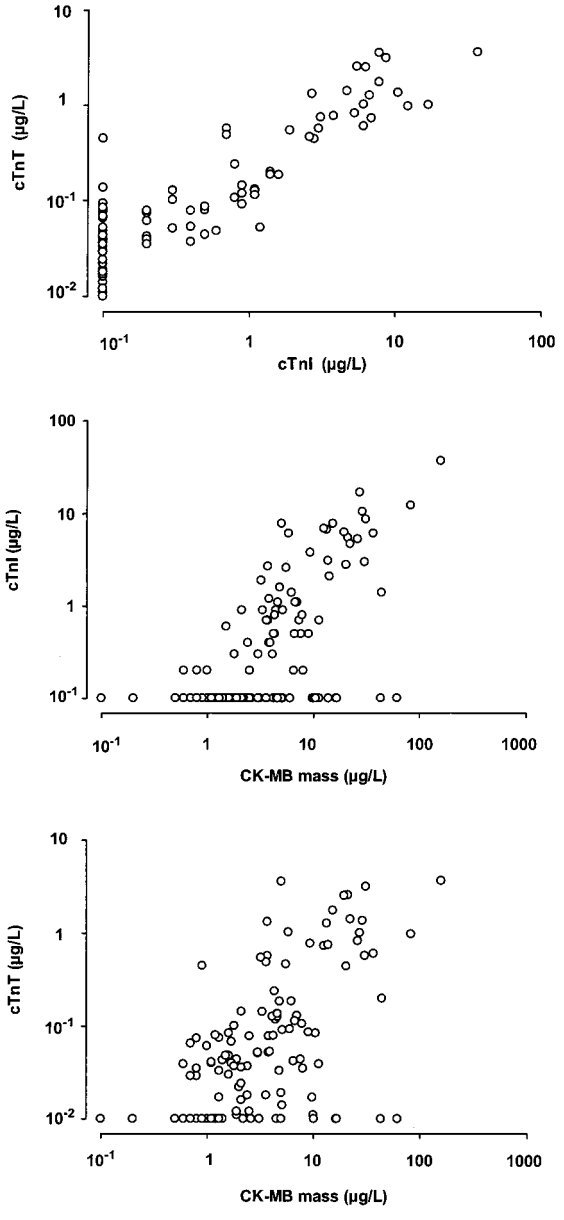

Fig. 1. Correlation between cardiac markers.

(*Top*), cTnI and cTnT (n = 116; $r = 0.847$; $P < 0.0001$); (*middle*), CK-MB mass and cTnI (n = 132; $r = 0.618$; $P < 0.0001$); (*bottom*), CK-MB mass and cTnI (n = 116; $r = 0.525$; $P < 0.0001$; Spearman correlation). All samples were taken within the first 48 h after inclusion.

ANATOMOPATHOLOGICAL FINDINGS

In nonsurvivors, the elapsed time from study entry to death averaged 8 days (range, 1–30 days). Multiorgan failure was the most common cause of death. Three patients died of a late cardiac event (Table 5). Autopsies were performed on seven cTnI-positive and five cTnI-negative subjects. Two of these patients died of a cardiac event. Postmortem investigation revealed a LV free wall rupture in a cTnI-negative patient who underwent prolonged cardiac massage and repeated electrical cardioversion after developing sudden ventricular fibrillation on day 3. Autopsy showed an extensive anterior myocardial infarction in a cTnI-positive patient who died from intractable cardiogenic shock on day 7 after inclusion. No postmortem investigation could be performed on a cTnI-positive patient who died of a cardiac arrest on day 3 without any sign of acute ischemia on ECG recordings. Microscopic examination of the heart excluded the presence of myocardial infarction in 10 other patients (6 cTnI positive and 4 cTnI negative) and documented the presence of similar aspecific myocardial changes in marker-positive and -negative patients. These included limited areas of elongated myocardial fibers, hypertrophied cardiomyocytes, and slight interstitial edema as well as small clusters of "wavy" myocytes with condensed sarcoplasm and preserved nuclei. Myocardial infarction and inflammatory cell infiltration could not be demonstrated. Contraction band necrosis was seen in three of six cTnI-positive patients (50%) and one of four cTnI-negative patients (25%; $P > 0.05$). Interstitial fibrosis was documented in one cTnI-positive patient with a decreased LV function and in one cTnI-negative patient with normal LV function. One cTnI-positive patient with abnormal LV function had interstitial amyloidosis.

Discussion

This study confirms and extends earlier observations on the high prevalence of increased cTn concentrations in immunocompetent patients with acute resuscitated septic shock *(23, 25, 26)*. In addition, this study demonstrates a highly significant association in this clinical situation between the presence of these specific and sensitive markers of myocardial cell injury *(7–24)* and LV dysfunction objectified by TEE. In our series of 46 patients, 50% displayed increased cTnI values at some time point during the first 48 h after admission to the ICU, in agreement with 68% (18 of 26) cTnT positivity reported in a previous smaller study by Spies et al. *(23)* on cardiac markers in early sepsis. In our hands, cTnI was a more sensitive marker than cTnT or CK-MB; nevertheless, the concentrations of these three cardiac markers were significantly correlated, supporting release from damaged myocytes as an explanation for the increased plasma concentrations, rather than spuriously increased test results. The observed differences in marker prevalences may derive from differences in diagnostic performance between the various assays used or from differences in stability, structure, myocyte content, and cellular release between the cardiac markers tested. The higher sensitivity of cTnI compared with cTnT contrasts with observations in other clinical situations *(15)*. Because different cTn isoforms are recognized to various degrees in certain assays, the possibility should be considered that different cTn fragments may be released in different pathophysiological circumstances *(37)*.

In agreement with previous observations in critically ill patients, cTn positivity was weakly associated with hospital mortality *(22, 23)*. Moreover, the temporal evolution of cTn concentrations did not contribute to a better assessment of poor outcome *(22)*. Clinically unrecognized

Table 4. Demographic, clinical, functional, and biological characteristics of cTnI-positive vs cTnI-negative patients with septic shock.

Characteristics	cTnI		*P*
	Positive[a] (n = 23)	Negative (n = 23)	
Demographic			
Male-to-female ratio	13/10	16/7	NS[b,c]
Age, median (range), years	71 (18–93)	56 (19–86)	0.001[d,e]
Clinical and functional			
APACHE II score, median (interquartile range)	27 (24–32)	20 (18–23)	0.0001[d,f]
Inotrope score, median (interquartile range)	5 (2–7)	4 (2–5)	NS[d]
Catecholamine dose,[g] median (interquartile range), $\mu g \cdot kg^{-1} \cdot min^{-1}$			
Dopamine	3.0 (0–7.5)	3.0 (0–10.0)	NS[d]
Dobutamine	10 (4–15)	5 (0–10)	NS[d]
Norepinephrine	0.1 (0.0–0.5)	0.1 (0.0–0.3)	NS[d]
Survival, n (%)	9 (39)	16 (69)	0.038[c,h]
Gram-negative pathogen, n (%)	13/19 (68)	16/20 (80)	NS[c]
Medical history, n (%)			
AMI	9 (39)	1 (4)	0.010[c,h]
Arterial hypertension	10 (43)	3 (13)	0.047[c,h]
ECG signs of ischemia, n (%)	0 (0)	0 (0)	NS[c]
LV dysfunction, n (%)	18 (78)	2 (9)	<0.0001[c,f]
Biological			
cTnT positivity,[a] n (%)	16/23 (70)	0/22 (0)	<0.0001[c,f]
CK-MB positivity, n (%)	12/23 (52)	7/23 (30)	NS[c]
Peak procalcitonin, μg/L	19.5	5.3	NS[d]
Median (interquartile range), μg/L	(2.8–45.5)	(1.3–23.2)	
Peak CRP, mg/L	245	284	NS[d]
Median (interquartile range), mg/L	(159–346)	(243–361)	

[a] cTnI ≥0.4 μg/L, cTnT ≥0.1 μg/L at one or more time points (at admission, and after 24 and 48 h).
[b] NS, not significant.
[c] Fisher's exact test.
[d] Mann–Whitney *U*-test.
[e] P_c <0.02 after correction for the number of comparisons (n = 17; Bonferroni adjustment).
[f] P_c <0.002 after correction for the number of comparisons (n = 17; Bonferroni adjustment).
[g] At the time of TEE.
[h] P_c not significant after correction for the number of comparisons (n = 17; Bonferroni adjustment).

cardiac damage, as expressed by increased cTn concentrations, is thus not a very potent predictor of hospital mortality, which may occur after the acute septic episode in some instances and may also be affected by other factors, such as age and underlying disease. We therefore used both univariate and multivariate approaches to investigate the clinical, functional, and biological correlates of increased cTnI and cTnT concentrations. Univariate analysis indicated that cTn positivity was closely associated with age, with clinical severity at diagnosis expressed by the APACHE II score, and with LV dysfunction as objectified by TEE. There was a tendency toward association with a history of AMI and arterial hypertension. However, multivariate analysis disclosed that all of these observed associations were secondary to the highly significant relationship between cTn concentrations and LV dysfunction. This observation further documents the value of cTnI and cTnT as specific and sensitive markers of minor myocardial damage *(10–15, 20–26)*.

Table 5. Causes of death in nonsurvivors according to cTnI status.

	Nonsurvivors, n (%)	cTnI	
		Positive[a,b]	Negative[b]
Multiorgan failure	11 (53)	9	2
Refractory shock	7 (33)	5	2
Cardiac death	3 (14)	2	1
All deaths	21 (100)	16	5

[a] cTnI ≥0.4 μg/L at one or more time points (admission, 24 and 48 h later).
[b] *P* >0.05 between cTnI-positive and -negative patients by Fisher's exact test.

The association between cTnI positivity and LV dysfunction observed in the present study was much stronger ($P < 0.0001$) than the recently published negative correlation between cTnI concentrations and LV stroke work index ($P < 0.01$) in a study of 15 patients with septic shock *(26)*. The latter report also showed a weak correlation ($P < 0.04$) between cTnI concentrations and maximum dose

of epinephrine/norepinephrine *(26)*. In the present study, no association between cTnI concentrations and inotrope score or specific dose of norepinephrine at the time of TEE was found. These differences may relate to the larger size of the present study and the fact that in septic shock, cardiac function can be more accurately assessed by TEE than by the previously used derived hemodynamic indicators *(26)*. In septic shock, TEE indeed enables a more precise preload estimation, particularly during mechanical ventilation; allows better dosing of inotropic support in relation to cardiac compliance; and offers a superior insight in cardiac morphology, valvular function, and concomitant right ventricular status *(32, 38)*.

Why circulating cTn concentrations are frequently increased during septic shock and whether such increases are causally related to the observed LV dysfunction or just represent consequences of—or changes not related to—the functional changes are issues that the present clinical observations are unable to solve. Continuous ECG monitoring and TEE examination at diagnosis did not disclose developing ischemia and excluded myocardial infarction during the first 2 days after inclusion in the study. In 12 nonsurvivors, an autopsy could be performed: in all but 1 patient (who died of AMI on day 7), there was no anatomopathological evidence of massive myocardial necrosis. Contraction band necrosis, an early marker of irreversible myocyte injury *(34, 39)*, was identified in 4 of the 10 nonsurvivors (without cardiac cause of death). In this small group, there was no significant difference in occurrence between cTnI-positive and -negative patients, although contraction band necrosis tended to be more frequent in cTnI-positive subjects. Contraction band necrosis has been associated with a large variety of conditions, including coronary occlusion and myocardial infarction; reperfusion following temporary ischemia; increased catecholamine concentrations, either exogenous or endogenous; intracranial hemorrhage; potassium or magnesium deficiency; defibrillation; and hemorrhagic shock *(34, 39)*. The condition is believed to result from an exaggerated flow of calcium into the myocardial fibers, which on turn cancels the troponin inhibition of actin and myosin interaction, allowing excess excitation-contraction coupling *(39)*. In the absence of contraction band necrosis, it is conceivable that small, nonsustained increases in cTnI may result from reversible injury to the sarcolemma, from diffuse irreversible damage leading to multiple foci of micronecrosis, or less probably, from apoptosis that may have been missed by the light microscopic observations in the nonsurvivors *(40, 41)*. It is indeed known that cTn isoforms can be released by the myocardial cells from a cytosolic store or from a myofibril-associated store *(40, 41)*.

Several factors may contribute to the occurrence of minimal myocardial damage during septic shock. A possible direct cardiac myocytotoxic effect of bacterial endotoxins or of local and circulating mediators (e.g., cytokines or reactive oxygen species) induced by the infectious process and produced by activated leukocytes, macrophages, and endothelial cells *(2)* should be considered. Alternatively, ischemia and reperfusion damage associated with microvascular dysfunction or resuscitation procedures (e.g., the use of vasopressors) could be involved. Aggressive inotropic treatment to boost systemic oxygen consumption increased the incidence of cardiovascular complications and adversely affected outcome in fluid-resuscitated septic patients *(42, 43)*. It is indeed conceivable that elaborate attempts at hyperresuscitation could either cause or disclose ischemic myocardial damage. However, no association between inotrope score, specific catecholamine dose, and cTn positivity was found in the present study. Finally, one should consider the possibility that increased cardiac filling pressures and increased wall stress may contribute to myocyte damage and micronecrosis as suggested for congestive heart failure *(40)*.

In conclusion, the present study documented a high prevalence of biochemical markers of cardiac injury in early septic shock. cTn positivity was significantly associated with echocardiographically objectified LV dysfunction. Increased cTn concentrations tended to occur more frequently in older patients with high APACHE II scores, poorer outcome, and a medical history of arterial hypertension or coronary artery disease, but these associations appeared secondary to the association with LV dysfunction in multivariate analysis. To refine the diagnostic use of cardiac markers and the therapeutic attitude toward cardiovascular alterations in septic shock, there is a clear need for more precise knowledge of the underlying pathophysiological relationships between causes and mediators of septic shock, LV dysfunction, and minor, clinically unrecognized cardiac damage. In this respect, animal models may provide better opportunities to study the exact nature and timing of events in relation to histological and ultrastructural changes in the myocardium.

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